'Aδ' fibres. The 'C' fibre nociceptors consist of the polymodal nociceptors, which respond to nociceptive heat, chemical and mechanical stimuli; and mechanical nociceptors, situated in subcutaneous tissue, which respond only to nociceptive mechanical stimuli. The 'Aδ' fibre nociceptors are mainly specific mechanical nociceptors (Burgess & Perl 1973; Iggo 1974). Neonatal capsaicin administration produces a large decrease in the number of 'C' fibres and has little effect on the 'Aδ' population. This may explain why the forceps pinch response, which is probably 'Aδ' mediated, is not affected by capsaicin; whereas the nociceptive response produced by the blunt point used in our experiments, which is probably 'C' fibre mediated, is altered. It is difficult to see why nociceptive heat thresholds are unchanged, as there must be a large decrease in the number of polymodal nociceptors. It is possible that the rat adapts to use the small number of remaining 'C' fibres to transmit nociceptive heat impulses, whereas input in a large number of fibres is necessary for transmission of nociceptive pressure and chemical impulses.

The fact that dorsal horn SP is depleted by neonatal capsaicin treatment (Gamse et al 1980; Nagy et al 1980) indicates that SP may mediate transmission of pressure and chemically-induced nociception at primary afferent terminals. However, the fact that heat nociception is apparently unaffected by such treatment indicates either that SP is not involved in transmission of nociceptive heat impulses or that there is a high safety factor for SP mediated transmission in this pathway.

The authors would like to thank Mrs Janine Thomson, Mrs Deborah Murray and Dr Alma Gower for technical assistance.

REFERENCES

Burgess, P. R., Perl, E. R. (1973) in: Iggo, A. (ed.) Handbook of Sensory Physiology, Vol. II, The Somatosensory System. Springer-Verlag, Berlin, Heidelberg, New York, pp 29–78

Gamse, R., Holzer, P., Lembeck, F. (1980) Br. J. Pharmacol. 68: 207–213

Hayes, A. G., Tyers, M. B. (1980) Brain Res. 189: 561–564

Hayes, A. G., Skingle, M., Tyers, M. B. (1980) Br. J. Pharmacol. 70: 96P

Henry, J. L. (1976) Brain Res. 114: 439–451

Hökfelt, T., Kellerth, J-O., Nilsson, G., Pernow, B. (1975) Ibid. 100: 235–252

Holzer, P., Jurna, I., Gamse, R., Lembeck, F. (1979) Eur. J. Pharmacol. 58: 511–514

Iggo, A. (1974) in: Bonica, J. J., Procacci, P., Pagni, C. A. (eds) Recent Advances in Pain: Pathophysiology and Clinical Aspects. C. C. Thomas, Springfield USA, pp 3–35

Irwin, S. (1968) Psychopharmacologia (Berlin) 13: 222–257

Jancsó, G., Kiraly, E., Jancsó-Gabór, A. (1977) Nature (London) 270: 741–743

Jessell, T. M., Iversen, L. L., Cuello, A. C. (1978) Brain Res. 152: 183–188

Jones, B. J., Roberts, D. J. (1968) J. Pharm. Pharmacol. 20: 302–304

Lawson, S. N., Nickels, S. M. (1980) J. Physiol. (London) 303: 19P

Nagy, J. I., Vincent, S. R., Staines, W. A., Fibiger, H. A., Reisine, T. D., Yamamura, H. I. (1980) Brain Res. 186: 435–444

Obal, F., Benedek, G., Jancsó-Gabór, A., Obal, F. (1979) Pflügers Arch. 382: 249–254

Randić, M., Miletić, V. (1977) Brain Res. 128: 164–169

Scadding, J. W. (1980) J. Anatomy 131: 473–484

J. Pharm. Pharmacol. 1980, 33: 185–187
Communicated July 1, 1980

0022–3573/81/030185–03 $02.50/0

Relationship between camazepam, *N*-methyl-oxazepam and oxazepam brain concentrations and antileptazol effect in the rat

S. Caccia*, M. Ballabio, S. Garattini, *Instituto di Ricerche Farmacologiche "Mario Negri" Via Eritrea, 62–20157 Milan, Italy*

Camazepam (7-chloro-3,*NN*-dimethylcarbamoyloxy-5-phenyl-1-methyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one) has the pharmacological profile of an anxiolytic agent with a clear separation between anxiolytic action and sedative-depressive side effects (Ferrini et al 1974).

Studies of the metabolism of camazepam indicate that the compound is excreted in the urine of several animal species, partly free and partly as *N*-methyl-oxazepam and oxazepam glucuronides (Garattini et al 1977). *N*-Methyl-oxazepam (Temazepam) and oxazepam are pharmacologically active metabolites (Marcucci et al 1968, 1972; Randall & Kappell 1973; Garattini et al 1973) and may be considered possible contributors to the central nervous system activity of the parent compound.

This hypothesis was investigated in the present experiments by comparing brain concentrations of *N*-methyl-oxazepam and oxazepam after administration of the two metabolites or of camazepam at doses effective against leptazol (pentetrazol) induced convulsions.

Male CD-Sprague Dawley rats (Charles River, Italy) 200 g were used. Benzodiazepines were administered orally, suspended in 0·5% carboxymethylcellulose, or injected intravenously dissolved in propylglycol-glycofurol-benzyl alcohol-water (30:30:2:48) at doses

* Correspondence.

Table 1. Brain concentrations of camazepam (CZ), *N*-methyl-oxazepam (MOX) and oxazepam (OX) in rats after administration of CZ at the ED50s on leptazol convulsions.

Time*	Route	ED50 (95% confidence limits) mg kg^{-1}	Brain concns (μg g^{-1} with s.d.)**		
			CZ	MOX	OX
5	i.v.	5·19 (7·03– 3·83)	3·49 (0·52)	<0·025	<0·025
30	oral	55·08 (73·54–41·30)	0·26 (0·06)	0·18 (0·04)	0·11 (0·03)
180	oral	33·65 (41·69–27·16)	0·24 (0·08)	0·21 (0·07)	0·12 (0·05)

* Min between CZ pretreatment and leptazol (120 mg kg^{-1} i.p.).
** Each value is the mean of 5 rats

Table 2. Brain concentrations of *N*-methyl-oxazepam (MOX) and oxazepam (OX) after administration of MOX or OX at the ED50s on leptazol convulsions.

Drugs	Time	Route	ED50 (95% confidence limits) mg kg^{-1}	Brain concns (μg g^{-1} with s.d.)**	
				MOX	OX
MOX	5	i.v.	0·18 (0·23–0·14)	0·24 ± 0·04	<0·025
	30	oral	4·12 (5·06–3·36)	0·15 ± 0·03	0·11 ± 0·03
	180	oral	10·33 (13·30–8·02)	0·17 ± 0·06	0·08 ± 0·01
OX	5	i.v.	0·21 (0·27–0·17)	—	0·23 ± 0·04
	30	oral	6·74 (9·46–4·81)	—	0·26 ± 0·07
	180	oral	10·30 (12·84–8·31)	—	0·27 ± 0·06

* Min between drug pretreatment and leptazol (120 mg kg^{-1}, i.p.).
** Each value is the mean of 5 rats.

corresponding to their antileptazol ED50, i.e. the doses (mg kg^{-1}) protecting 50% of the rats from convulsions elicited by leptazol (120 mg kg^{-1} i.p.). Each ED50 was calculated, in separate experiments, on at least 5 dose levels with 8 rats for each dose. Brain concentrations of camazepam and its metabolites were determined by a g.l.c. method according to Marcucci et al (1978) with minor modifications.

After administration of camazepam at the ED50 of leptazol, the active brain concentrations (50% inhibition of leptazol-induced convulsions) of the compound varied depending on the route and time of administration (Table 1). Camazepam brain concentrations of about 3·5 μg g^{-1} were necessary in the rat to ensure significant protection after intravenous injection, while brain concentrations (about 0·25 μg g^{-1}) were lower after oral administration of the drug. At the intervals considered, both *N*-methyl-oxazepam and oxazepam were present in the brain of orally-treated rats while they were undetectable (< 0·025 μg g^{-1}) 5 min after intravenous injection. The results as far as the intravenous route is concerned are in agreement with previous data of Marcucci et al (1978). This suggests that after oral administration, at the times considered, the metabolites contribute to the anticonvulsant effect of the parent compound.

To prove this, and to see how much the metabolites contribute to the anticonvulsant effect, in another experiment we measured the active brain concentrations of *N*-methyl-oxazepam and oxazepam after their administration at the ED50 on leptazol convulsions. Table 2 shows that methyl-oxazepam and oxazepam were both extremely active and equally potent in terms of dosage and brain concentrations (≈ 0·25 μg g^{-1}) necessary to antagonize leptazol-induced convulsions. When administered orally *N*-methyl-oxazepam reached brain concentrations lower than 0·25 μg g^{-1}. At these intervals 0·1 μg g^{-1} of oxazepam was also present in the rat brain and may have contributed to the protective effect of *N*-methyl-oxazepam. The brain concentrations of *N*-methyloxazepam and oxazepam in these experiments were virtually the sum of the concentrations of the metabolites found after oral administration of camazepam, so the pharmacological effect is probably mediated by both active metabolites.

In conclusion these findings indicate that the antileptazol activity of oral camazepam in the rat is dependent more on the brain concentrations of active metabolites than on the parent compound. However, these conclusions only concern the antileptazol activity of camazepam in the rat. Further studies are needed to see how much the metabolites contribute to other pharmacological effects of camazepam.

REFERENCES

Ferrini, R., Miragoli, G., Taccardi, B. (1974) Arzneim. Forsch. 24: 2029–2032

Garattini, S., Marcucci, F., Mussini, E. (1977) in: Usdin, E., Forrest, I. S. (eds) Psychotherapeutic Drugs, Part II. Applications. M. Dekker, New York, pp 1039–1087

Garattini, S., Mussini, E., Marcucci, F., Guaitani, A. (1973) in: Garattini, S., Mussini, E., Randall, L. O. (eds) The Benzodiazepines. Raven Press, New York, pp 75–97

Marcucci, F., Guaitani, A., Kvetina, J., Mussini, E., Garattini, S. (1968) Eur. J. Pharmacol. 4: 467–470

Marcucci, F., Mussini, E., Airoldi, L., Guaitani, A., Garattini, S. (1972) J. Pharm. Pharmacol. 24: 63–64

Marcucci, F., Mussini, E., Cotellessa, L., Ghirardi, P., Parenti, M., Riva, R., Salvà, P. (1978) J. Pharm. Sci. 67: 1470–1471

Randall, L. O., Kappell, B. (1973) in: Garattini, S., Mussini, E., Randall, L. O. (eds) The Benzodiazepines. Raven Press, New York, pp 27–51

J. Pharm. Pharmacol. 1981, 33: 187–188
Communicated November 5, 1980

0022–3573/81/030187–02 $02.50/0

α_2-Adrenoceptor-blocking action of the phenylethanolamine-*N*-methyltransferase inhibitor SKF 64139

G. M. DREW, *Department of Pharmacology, Glaxo Group Research, Ware, Hertfordshire, SG12 0DJ, U.K.*

7,8-Dichloro-1,2,3,4-tetrahydroisoquinoline (SKF 64139) is an inhibitor of phenylethanolamine-*N*-methyltransferase (PNMT–Pendleton et al 1976). When administered to rats, it induced the depletion of adrenaline from the dorsal midline area of the caudal medulla oblongata, and this effect was significantly reduced by clonidine (Fuxe et al 1979). In addition to its ability to inhibit PNMT, SKF 64139 is a weak, but specific, antagonist at the postsynaptic α_1-adrenoceptors in rabbit aorta ($pA_2 = 5{\cdot}2$; Pendleton et al 1976). In view of this observation it seemed possible that SKF 64139 might also be an antagonist at presynaptic α_2-adrenoceptors. The experiments now reported were carried out to investigate this possibility.

The pA_2 for SKF 64139 against noradrenaline (Koch-Light) at post-synaptic α_1-adrenoceptors in rabbit isolated aorta was determined as described by Apperley et al (1976). The pA_2 for SKF 64139 against clonidine (Boehringer) at presynaptic α_2-adrenoceptors in the guinea-pig isolated ileum was determined using the method described by Drew (1978).

SKF 64139 (10^{-5}, 3×10^{-5} and 10^{-4} M) caused concentration-dependent, parallel, rightward displacements of the concentration-response curve to noradrenaline in the rabbit aorta without causing any reduction in the maximum response to noradrenaline. Similarly SKF 64139 (10^{-6}, 3×10^{-6} and 10^{-5} M) antagonized the clonidine-induced inhibition of the twitch response to field stimulation of the guinea-pig ileum, although it was rather more potent an antagonist in this tissue. SKF 64139, itself, had little or no effect on the twitch response and did not reduce the maximal response to clonidine. The mean pA_2 values for SKF 64139 at α_1- and α_2-adrenoceptors, and the mean slopes of the Schild plots from which they are derived, are shown in Table 1. It can be seen that SKF 64139 is approximately twenty times more potent at blocking effects mediated via α_2- than via α_1-adrenoceptors in vitro. The α_1:α_2 potency is similar to that found for yohimbine (Doxey et al 1977), but SKF 64139 is approximately 10 times less potent than yohimbine.

Table 1. The α-adrenoceptor-blocking potencies of SKF 64139. Results are expressed as mean (and 95% confidence limits). Slope = Slope of regression of log (agonist dose-ratio-1) vs log molar concentration of antagonist (Arunlakshana & Schild 1959).

	Postsynaptic α_1-adrenoceptors (rabbit aorta) (n = 6)	Presynaptic α_2-adrenoceptors (guinea-pig ileum) (n = 7)
pA_2	5·47 (5·24–5·70)	6·79 (6·60–6·98)
Slope	0·98 (0·88–1·08)	1·17 (1·03–1·31)

n = Number of experiments.

In view of its relatively high potency in blocking α_2-adrenoceptors it is possible that this effect of SKF 64139 will occur at plasma concentrations shown to inhibit PNMT (Pendleton et al 1976). Thus, caution should be exercised when interpreting the interaction between SKF 64139 and α-adrenoceptor agonists, or the effects of SKF 64139 on adrenaline turnover, since presynaptic α_2-adrenoceptor blockade or PNMT inhibition could produce similar effects. For example, either effect of SKF 64139 could lead to a clonidine-sensitive increase in adrenaline turnover (Scatton et al 1979). In the central nervous system some α_2-adrenoceptors, such as those in the locus coeruleus, are located postsynaptically (Cedarbaum & Aghajanian 1977) and may receive an inhibitory adrenergic input; the SKF 64139-induced acceleration in the firing rate of locus coeruleus units observed by Svensson & Engberg (1980) could be due either to its PNMT-inhibitory activity or to blockade of these postsynaptic α_2-adrenoceptors. Other PNMT inhibitors related to SKF 64139 should be investigated for their α-adrenoceptor-blocking properties.